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Prevalence and Mother-to-Infant Transmission of Hepatitis Viruses B, C, and E in Southern Tanzania

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Hepatitis B and C markers were tested in 980 pregnant women, in the infants born to infected mothers, and in a random sample of 42 and 50, respectively, children born to uninfected mothers in Tanzania. Sixty-two women (6.3%) were positive for HBsAg and 15 (24%) were HBeAg-seropositive. Anti-HCV was detected in 49 women (5%), 15 (31%) of whom had detectable viremia. HCV RNA serum levels were low and only genotype 4 was identified. Sixty-six women (6.7%) were positive for anti-HIV, six of whom were coinfected with HBV and one with HCV. Anti-HEV was negative in the 180 women tested. At 8 months of age, HBsAg was detected in 8% and 2% of children born to HBV-infected and noninfected mothers, respectively ($P = 0.2$). Corresponding figures at 18 months of age were 31% and 21% ($P = 0.3$). When tested at 2 months of age, HCV RNA was not detected in any of the 43 children born to anti-HCV–positive mothers nor in any of 50 children born to anti-HCV–negative mothers. At 18 months, only one child, born to an anti-HCV–positive mother, had detectable HCV RNA. None of the infants born to women with HIV coinfection were infected with hepatitis viruses. This study suggests that exposure to HEV does not occur in southern Tanzania. The prevalence of current HBV infection in pregnant women from rural Tanzania is lower than in other sub-Saharan areas. In early childhood, HBV infection appears to occur by horizontal rather than maternofilial mechanisms of transmission. The prevalence of HCV infection is similar to that in other African countries. The results of this study show for the first time in Africa that mother-to-infant transmission does not play a significant role in the acquisition of HCV infection. ***J. Med. Virol. 58:215–220, 1999.***

KEY WORDS: maternal; Africa; vertical transmission; viral hepatitis

INTRODUCTION

Viral hepatitis is a recognized major public health problem worldwide, but its impact is greater in the least economically favored areas. Of the known hepatitis viruses, hepatitis B virus (HBV) and C virus (HCV) have received more attention due to their wide distribution and high prevalence, and also to the severe long-term consequences of chronic infections with these agents [Di Bisceglie et al., 1988; Alter et al., 1992].

The prevalence of serological markers of exposure to HBV in sub-Saharan Africa is high, up to 90% in many areas [Ayoola, 1988]. The prevalence of HBV carriers varies substantially between regions, from less than 7% to 35% [Bowry, 1983; Stahel et al., 1984; Triki et al., 1997]. Variations in the composition of the populations surveyed as well as on the frequency of both environmental and genetic risk factors might explain these differences. Perinatal transmission of HBV accounts for the majority of childhood HBV infections in Asia

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[Chen et al., 1996], but this form of transmission seems to play a minor role in Africa [Goudeau et al., 1983; Whittle et al., 1983]. This is mainly due to the lower prevalence of HBeAg positivity among African pregnant women, indicating lower viral replication [Harry et al., 1994]. However, the possible role of perinatal transmission should be considered when studying the epidemiology of HBV.

Prevalence studies on HCV infection have been reported from several areas in Africa [Schoub et al., 1992; Xu et al., 1994; Arthur et al., 1997]. Early studies using first-generation tests reported a very high prevalence of HCV antibodies, but these results were not confirmed with more specific assays [Tibbs et al., 1991]. The risk of HCV infection following parenteral exposure is well established [Alter et al., 1990]. There is more conflicting evidence on the role of other modes of transmission such as sexual and nonparenteral. Mother-to- infant transmission (either in utero or at birth) has been reported in several studies from Europe and Japan, but the results were conflicting [Ohto et al., 1994; Zanetti et al., 1995; Sabatino et al., 1996]. A recent review concluded that mother-to-infant transmission of HCV is mostly restricted to infants born to HCV highly viremic mothers, which in turn is related to the HIV status of the woman. The risk of transmission is generally low, less than 10% in HIV-negative women [Thomas et al., 1997]. Little information is available from Africa concerning mother-to-infant transmission of HCV. This may be particularly relevant in areas where HIV is a significant problem.

Previous studies found a 4% prevalence of HBV infection in Tanzanian pregnant women [Pellizer et al., 1994], but there is no information concerning the risk of mother-to-infant transmission of HBV. On the other hand, epidemiological data on HCV and HEV infections are not available from this country. Given that HIV infection has been associated with increased vertical transmission of HCV and that it constitutes a growing problem in sub-Saharan Africa, we have also assessed the prevalence of anti-HIV in these group of women. We report the results of a descriptive study on the prevalence of HBV, HCV, and HEV infections and the risk of mother-to-infant transmission in a group of unselected pregnant women living in a rural area of Tanzania.

MATERIALS AND METHODS

Study Area and Population

The study was carried out in the town of Ifakara, Kilombero District, Morogoro region of southeastern Tanzania. The population of Ifakara is estimated at 70,000 and has been growing considerably in recent years. Most villagers are subsistence farmers growing rice and maize but there is also an increasing number of small traders.

The town is served by a 375-bed district hospital (St. Francis Designated District Hospital, SFDDH) and the adjacent Maternal and Child Health Clinic (MCH). More than 60% of pregnant women from Ifakara deliver at SFDDH. Blood donors are screened for HIV antibodies only.

On admission to the hospital for delivery, a copy of the consent letter in Kiswahili, which included detailed information on procedures and potential risks and benefits of the study, was given to the mothers. This letter was then read by a project medical assistant who would answer any questions. After checking with preestablished key questions that the mother had understood all implications, she was invited to sign the consent form.

The study was carried out as part of a trial on the prevention of malaria and anemia in infants (IRONMAL study), whose research clearance was granted by the Medical Research Coordinating Committee of the National Institute for Medical Research through the Tanzanian Commission for Science and Technology as per reference 90/167/3049/94.

Study Design

This study was undertaken in conjunction with a trial of prevention of malaria and anemia in infants [Menendez et al., 1997]. During a 10-month period (January to October 1995), all women resident in Ifakara who delivered at SFDDH and gave their informed consent were enrolled; 980 women and their 985 infants were recruited into the study. At delivery, maternal blood samples were collected into EDTA tubes for hematological assessment. Aliquots of plasma were separated and stored frozen at –20°C. The birth weight of the baby was recorded and the gestational age assessed.

HBsAg, anti-HBc, anti-HCV, and anti-HIV were examined in all women. HBeAg, anti-HBe, and HBV DNA were tested in HBsAg-positive women, and anti-HBs were determined in anti-HBc–positive cases. All samples positive for anti-HCV were tested for HCV RNA and retested for anti-HCV with a supplementary assay. Anti-HEV was determined in 180 women randomly selected.

Infants were followed up until 18 months of age. Cross-sectional surveys were carried out at 2, 5, 8, 12, and 18 months of age. At each visit, a finger prick blood sample was collected for hematological and parasitological determinations and the plasma stored at –20°C or below. HBsAg was determined in children born to HBV carrier mothers and in a random sample of infants born to negative mothers for both HBV and HCV infection. Anti-HCV was determined and HCV RNA analyzed in the infants born to mothers positive for anti-HCV and in a random sample of children born to negative mothers for both HBV and HCV infection.

Laboratory Methods

All tests for hepatitis B (HBV), C (HCV), and E virus (HEV), as well as anti-HIV, were carried out at the Hospital Clinic in Barcelona, Spain. HBsAg was sought with a commercial ELISA kit from Ortho Diagnostics Systems. The presence of hepatitis B e antigen (HBeAg), antibodies to hepatitis B surface antigen, e

and core antigens (anti-HBs, anti-HBe, and anti-HBc, respectively) were determined by commercial ELISA kits from Sorin Biomedica (Saluggia, Italy). HBV DNA was extracted from 140 μl of plasma using commercially available columns (QIA amp HCV kit, Qiagen, Germany) and analyzed by nested PCR using primers from the precore-core region, which allows detection of DNA from both wild-type and precore mutants. The detection limit of the PCR technique was about 10 copies/mL of HBV DNA. Antibodies to HCV (anti-HCV) were tested using a commercial third-generation ELISA from Ortho Diagnostics Systems (Ortho HCV 3.0 ELISA). Reactive samples were tested by a recombinant immunoblot assay as a confirmatory test RIBA third-generation (Chiron RIBA HCV test systems 3.0, SIA). HCV RNA was determined in all reactive cases to the ELISA test by nested PCR using primers from the 5' noncoding region that allows detection of 200 copies/mL. HCV viremia was quantified by a modified competitive RT-PCR [Olmedo et al., 1999]. HCV genotype was determined by RFLP [Davidson et al., 1995].

Antibodies to HEV (anti-HEV) were detected using a commercial ELISA from Abbott (Abbott Diagnostics, North Chicago, IL). Antibodies to human immunodeficiency virus (HIV) were detected by Ortho HIV EIA (Raritan, NJ).

Statistical Analysis

Differences in prevalences were analyzed using the chi-square test and Fisher's exact test. The analysis was carried in EPI-Info version 6.01 (Center for Disease Control, Atlanta, GA).

RESULTS

Prevalence of Infection With Hepatitis Viruses B, C, and E in Mothers

Mean maternal age and parity was 24.5 (SD, 6.2; range, 15–50) and 3.3 (SD, 2.3; range, 1–11), respectively. HBsAg was detected in 6.3% (62) of the 980 women tested. HBeAg was detected in 12 of the 62 HBsAg-positive cases (19.3%), anti-HBe in 37 (59.6%), both HBeAg and anti-HBe in 3 (4.8%), and none of these markers in the remaining 10 (16.1%). Anti-HBc was detected in 551 (56.2%) women. Of them, 339 (61.5%) also showed anti-HBs positivity. In 162 women (16.5%) only anti-HBc was detected. HBV DNA was found in 10 (16.1%) of the 62 seropositive women, of whom 8 were also positive for HBeAg.

Anti-HCV was detected in 49 women (5%) by ELISA. The RIBA was positive in 23, indeterminate in 16, and negative in 10, thus the true minimal prevalence of anti-HCV was 2.3%. HCV RNA was sought in the 49 ELISA-positive women and the PCR test was positive in 15 (30.6%; Table I). The level of viremia in these mothers ranged between 5×10^3 and 66×10^4 copies per milliliter. Infection with genotype 4 was detected in the seven cases where genotyping studies were feasible. Two cases were coinfected with HBV.

Anti-HIV was positive in 66 mothers (6.7%). Of these, six were coinfected with HBV (HBsAg-positive) and only one was coinfected with HCV (anti-HCV– and HCV RNA-positive). HIV coinfection appear to be more frequent in HBV carriers (6/62, 9.7%) than in HCV positive women (1/49, 2.0%) (Fisher's exact test = 0.13), although the difference was not statistically significant. HBV DNA was detected in 1 out of 6 anti-HIV– and HBsAg-positive mothers (16%) and in 9 of 56 HBsAg-positive/anti-HIV–negative women (16%). Anti-HEV was not detected in any of the 180 women randomly selected for the study.

TABLE I. RIBA and HCV RNA Results in the 49 Mothers Positive for Anti-HCV by ELISA

RIBA	N	HCV RNA-positive	HCV RNA-negative
Positive	23	8	15
Indeterminate	16	6	10
Negative	10	1	9
Total	49	15	34

Transmission of Hepatitis Viruses to Infants

Fifty-three of the 62 children born to HBV carrier mothers were tested for HBsAg. Samples from the remaining children were not available due to migration (two), death (five), or refusal (two). HBsAg was determined at 8 and 18 months of age in 34 children, at 8 months only in 14, and at 18 months only in 5. Forty-two randomly selected children, born to mothers negative for both HBV and HCV markers, were also tested for HBsAg at 8 and 18 months of age.

Out of the 48 infants born to HBV carrier mothers tested at 8 months of age, 4 (8.3%) were positive for HBsAg, compared to 1 of the 42 children (2.4%) born to seronegative mothers (OR = 3.7; 95% CI: 0.3–91.3; Fisher's exact test; $P = 0.2$). At 18 months of age, 12 children out of the 39 (30.7%) born to HBsAg-positive mothers were HBsAg-seropositive, compared to 9 of the 42 (21.4%) born to HBsAg-negative mothers (OR = 1.6; 95% CI: 0.5–5.0; $\chi^2 = 0.9$, 1 df; $P = 0.3$). HBsAg remained positive at 18 months in three of the four infants who were HBsAg-seropositive at 8 months. These three children were born to mothers positive for HBeAg, whereas the child who became HBsAg-seronegative was born to a mother positive for anti-HBe and negative for HBeAg. HBsAg became detectable at 18 months of age in nine children in whom HBsAg was negative at 8 months, six were born to mothers positive for anti-HBe (Table II). Although the difference did not reach statistical significance, the risk of being HBsAg-positive at any time appear to be higher among the children born to HBeAg-positive mothers (6/15; 40.0%) than among those born to HBV carriers but HBeAg-negative women (7/38, 18.4%; OR = 2.9; 95% CI: 0.7–13.5; $P = 0.19$; $\chi^2 = 2.6$, 1 df; $P = 0.1$). The proportion of infants who became infected with HBV was similar in children born to women positive for anti-HBc alone and in those born to anti-HBc–negative mothers (14/53, 26.4% vs. 10/60, 16.6%; $\chi^2 = 1.6$, 1 df; $P = 0.2$). No relationship was found between

TABLE II. HBsAg Antigenemia at 8 and 18 Months of Age According to the Maternal HBV Status at Delivery

Maternal serologic status	Number of children tested	Number of children HBsAg-positive	
		At 8 months	At 18 months
HBsAg-positive/HBeAg-positive[a]	15	3	6[b]
HBsAg-positive/anti-HBe-positive[a]	38	1	6[c]
HBsAg-negative	42	1	9[c]
Total	95	5	21

[a]Odds ratio of being HBsAg-seropositive at 8 months of age in children born to seropositive mothers (OR = 3.7; 95% CI: 0.3–91.3). Odds ratio of being HBsAg-seropositive at 18 months of age in children born to seropositive mothers (OR = 1.6; 95% CI: 0.5–5.0).
[b]HBsAg was detected at 8 months of age in three of these six children.
[c]HBsAg was not detected in these children when tested at 8 months of age.

the risk of HBV infection in children, either at birth or later, and factors such as gestational age, birth weight, or breast feeding (data not shown).

Fifty babies were born to the 49 mothers positive for anti-HCV. Five children were not followed up due to death (two children) or migration (three children). The analysis is therefore referred to the 45 babies of the 44 mothers who remained under surveillance. Anti-HVC was determined at 18 months of age in 37 children, and at 7 to 12 months of age in 8 infants. HCV RNA was analyzed in 35 children at 2 and 18 months of age, in 2 infants at 18 months only, and in 8 children at 2 months of age only. HCV RNA and anti-HCV were tested at 2 and 18 months of age in 50 randomly selected infants born to mothers seronegative for HCV and HBV.

Out of the 37 children tested at 18 months of age, 2 were positive for anti-HCV by ELISA, but RIBA was negative. Anti-HCV was positive in two of the eight infants tested at 7 months, but this antibody became negative upon retesting 2 months later. RIBA was positive and the HCV RNA negative in the mothers of these two children. The child born to the woman coinfected with HIV and HCV was anti-HCV–seronegative in two samples taken at 2 and 11 months of age. HCV RNA was negative in the 43 infants tested at 2 months of age but it became positive in 1 of them when tested at 18 months of age. Anti-HCV and HCV RNA were both negative in all the samples analyzed from the 50 children born to HCV-seronegative mothers.

DISCUSSION

This study was done in connection with the intervention trial on the prevention of malaria and anemia in infants [Menendez et al., 1997], which consequently determined the sample size and follow-up of the cohort in the present study.

The prevalence of current HBV infection among unselected pregnant women from Ifakara, Tanzania, included in this study was lower than that reported from other areas of sub-Saharan Africa [Ndumbe et al., 1992; Roingeard et al., 1993] and similar to that from northern Africa [Hyams et al., 1988]. This study was not biased by the selective effect of targeting blood donors or risk groups from the community [Tibbs, 1997]. Therefore, it is likely that the observed 6% prevalence of HBs Ag-positivity adequately reflects the frequency of HBV carrier status in this community, as these women did not belong to a particular risk group for the infection. The prevalence of HBe antigenemia in HBsAg-seropositive mothers was 24%, which is similar to that found in other areas in Africa [Roingeard et al., 1993], but lower than that reported from Asia [Lin et al., 1994; Edmunds et al., 1996]. The prevalence of anti-HBc as a sole marker of HBV infection was 16.5%, which is similar to other areas of sub-Saharan Africa [Coursaget et al., 1984]. The results confirm that the epidemiological pattern of HBV infection varies between different regions.

The risk of vertical or perinatal mother-to-infant transmission of HBV appeared to be low. Only 3 of the 53 (5.6%) infants born to HBsAg-seropositive mothers were HBsAg-seropositive at 8 months of age and remained positive at 18 months of age. This figure is likely to represent the true rate of maternal transmission of HBV infection. In contrast, 18 of the 21 (85.7%) children seropositive at 18 months of age were HBsAg-seronegative when tested at 8 months. The risk of infection with HBV between 8 and 18 months of age was similar for children born to HBsAg-positive or HBsAg-negative mothers. This observation suggests that horizontal transmission is the most common mechanism of HBV infection in early childhood in this area.

There is little information concerning the prevalence of HCV infection in pregnant women from Africa. The ELISA anti-HCV was positive in 5% of the women studied. This prevalence is higher than that reported from studies among unselected pregnant women in Europe [Salleras et al., 1994; Zanetti et al., 1995] and similar to that found in previous studies from African countries such as Egypt, Cameroon, and Guinea Conakry [Hassan and Kotkat, 1993; Ndumbe and Skalsky, 1993; Ruggieri et al., 1996].

RIBA was positive in only 23 of the 49 (47%) anti-HCV ELISA-positive samples. This may indicate a high rate of false positive results of the ELISA test. However, RIBA may not be sensitive enough to confirm the results of the ELISA when studying samples from populations that include a large number of subjects infected with HCV genotypes other than genotype 1 or

genotype 2 [Dow et al., 1996; Zein et al., 1997]. Data from this study suggest that infection with genotype 4 is highly prevalent in Tanzania, as in other African countries [Xu et al., 1994; Smuts and Kannemeyer, 1995; Oni and Harrison, 1996]. This may explain the low rate of positivity of RIBA in anti-HCV–positive samples by ELISA. In fact, HCV RNA was detected by a highly sensitive and specific RT-nested PCR in almost a half of the anti-HCV ELISA-positive samples that were indeterminate or negative by RIBA (Table I).

HCV RNA was detected in only one-third of the women with detectable antibodies. The level of HCV viremia was generally low, having all less than 10^6 copies/ml of HCV RNA. There was a low rate of positivity for HCV RNA and a low level of HCV viremia in this study. It could be argued that this could have been related to a inadvertent problem in the preservation of samples. This is considered unlikely, since in parallel studies using the same samples a high prevalence of positivity of GBV-C/HGV RNA was found (data not shown). The latter suggests that the preservation of nucleic acid material in the samples under study was adequate, at least for qualitative HCV RNA studies.

To our knowledge, these are the first results on the risk of mother-to-infant transmission of HCV in Africa. Previous reports on the prevalence of HCV infection are based on nonlinked cohorts of pregnant women and children [Hassan and Kotkat, 1993; Ndumbe and Skalsky, 1993; Ruggieri et al., 1996], while our study is based on mother-infant pairs. A positive reaction for HCV RNA and anti-HCV was detected in only one child at 18 months of age. HCV RNA was negative when the child was tested at 2 months of age and his mother was anti-HCV–positive but HCV RNA-negative at delivery. Thus, clear-cut cases of mother-to-infant transmission of HCV infection were not observed in this study, confirming that HCV is less readily transmitted from mother to infant than HBV. It is currently believed that HCV transmission is largely restricted to infants born to viremic mothers [Thomas et al., 1997], and that the level of maternal viremia directly correlates with the risk of transmission to the infant. The low level of HCV viremia in HCV RNA-positive mothers may explain the absence of mother-to-infant transmission in our population.

The prevalence of infection with HIV was similar to that in other rural areas of southern Tanzania (8.7%) [Petry and Kingu, 1996] and lower than that reported from the capital Dar es Salaam (15.2%) [Mwakagile et al., 1996]. The frequency of coinfection with HIV was higher among HBsAg-positive than among HCV-positive mothers, although the difference did not reach statistical significance. Sharing a common route of transmission for HIV and HBV, probably sexual, and a different way for HCV, possibly parenteral, might explain this difference, although further epidemiological research is clearly warranted. The small number of anti-HIV–positive mothers coinfected with HBV or HCV did not allow us to draw any conclusions on the role of HIV coinfection in the risk of mother-to-infant transmission of hepatitis viruses.

None of the 180 women tested presented detectable anti-HEV antibodies. Absence or a low prevalence of anti-HEV has been reported in seroprevalence studies from Senegal and from Venezuela [Pujol et al., 1994; Coursaget et al., 1995]. This may be explained by a short-lived immunity to previous infection, with rapid clearance of specific antibodies [Skidmore, 1997]. Acute HEV hepatitis is largely confined to the developing world. It affects mainly adults, despite the well-recognized transmission through the fecal-oral route of this agent, that would result in infection and asymptomatic disease early in childhood [Hall, 1996]. However, lack of previous exposure to the infection or low sensitivity of the test under use may be alternative explanations to the absence of anti-HEV antibodies noted in this survey.

In conclusion, further studies on the prevalence of HCV infection in patients with liver diseases are needed to understand the impact of HCV infection in this population. On the other hand, although the risk of mother-to-infant transmission of HBV infection is low in this area, a control strategy of vaccination at or early after birth may be the most cost-effective recommendation to prevent HBV infection.

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